Clinical & Experimental Ophthalmology
RANZCO The Royal Australian and New Zealand College of Ophthalmologists
Clinical and Experimental Ophthalmology 2018; 46: 750–756 doi: 10.1111/ceo.13191

Original Article

Effect of subconjunctival Bevacizumab injection on the outcome of Ahmed glaucoma valve implantation: a randomized control trial

Arezoo Miraftabi MD, Naveed Nilforushan MD, Mina Darghahi MD, Sayyed Amirpooya Alemzadeh MD, Mohammad Parsamanesh MD and Maryam Yadgari MD
Eye Research Center, Rassoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

Abstract

Importance: The effect of subconjunctival Bevacizumab injection on the outcome of Ahmed glaucoma valve (AGV) implantation.

Background: Evaluation of efficacy and safety of subconjunctival Bevacizumab injection adjunctive to AGV implantation.

Design: Prospective and randomized clinical trial.

Participants: Fifty eyes of 50 patients with diagnosis of glaucoma that were candidate for AGV surgery were included.

Methods: In 25 eyes, conventional AGV surgery (group 1) and in 25 eyes AGV surgery with subconjunctival Bevacizumab (group 2) was performed by block randomization

Main Outcome Measures: The primary outcome measure was surgical success. Outcome measures were compared at postoperative month 3, 6 and 12.

Results: Mean age of patients was 58.76 ± 12.11 and 51.36 ± 15.44 years in group 1 and 2 respectively ($P = 0.06$). Mean intraocular pressure (IOP) at baseline was 24.88 ± 7.62 mmHg in group 1 and 27.52 ± 8.57 mmHg in group 2 which decreased to15.4 ± 4.4 mmHg in group 1 and 13.42 ± 2.9 mmHg in group 2 ($P < 0.00$) at last follow up. Surgical success was defined in two level: postoperative IOP ≤ 21 mmHg with at least 20% reduction in IOP (Criterion A), either with no medication (complete success) or with no more than two medications (qualified success) and criterion B with the same definition but the IOP ≤ 18 mmHg The cumulative success according to criterion A and B was 77.8%, 72.2% in group 1 and 89.5% in group 2, respectively, at the end of follow-up.

Conclusions and Relevance: Subconjunctival injection of Bevacizumab adjunctive to AGV implantation leads to higher success rate compared with AGV alone in one year follow-up.

Key words: Ahmed glaucoma valve, Bevacizumab, randomized control trial, subconjunctival injection.

Introduction

The role of aqueous shunts in modern glaucoma surgery has been widely increased since the publication of Tube versus Trabeculectomy Study.[1]

Although a lot of studies evaluated the role of antifibrotic agents such as mitomycin C (MMC) and 5-fluorouracil (5-FU) in trabeculectomy, there is

Correspondence: Dr Arezoo Miraftabi, Eye Research Center, Rassoul Akram Hospital Iran University of Medical Sciences, Tehran, Niayesh Street, Satarkhan Street, 1445613131, Iran. Email: arezoomiraftabi@yahoo.com

Received 24 October 2017; accepted 8 March 2018.

Conflict of interest: None declared.

Funding sources: None declared.

This study has been registered at Iranian Registry of Clinical Trials (Reg. No. Irct2016042527595N1), with the name of 'Effects of subconjunctival Bevacizumab in Ahmed Glaucoma Valve Surgery'.

little evidence on their role in shunt surgeries.[2]These agents have increased the success of trabeculectomy especially in patients with poor prognosis; but at the same time they have also increased the risk of late-onset complications, such as bleb leaks, hypotony and endophthalmitis.[2–6]There is large number of evidence on the role of anti-VGEF agents in reducing scar formation after glaucoma surgery, but the effect of anti-VEGF agents on the shunt surgeries is not clear yet.[7]

The success rate for tube shunts controlling various types of refractory glaucoma has been reported to be more than 50% in most previous series with different success criteria and lengths of follow-up.[1] Fibrovascular ingrowth into the valve chamber and dense encapsulation around the plate are among a probable causes of Ahmed glaucoma valve (AGV) failure.[7] Vascular endothelial growth factor (VEGF), which is an endothelial cell-specific mitogen and an angiogenic and also a fibroblast proliferation inducer, found to be an important stimulus for wound healing.[8–11] Bevacizumab (Bevacizumab; Genentech, San Francisco, CA, USA) is a humanized, non-selective monoclonal antibody against VEGF that commonly used in the ocular disease with a vascular component.[7] Studies showed that in patients with diagnosis of glaucoma and especially in neovascular glaucoma (NVG), VEGF concentration is increased in the aqueous humour.[12] The elevated VEGF levels in the aqueous humour can trigger fibrosis, [13] in the tissue around the plate when the aqueous reaches to the subconjunctival spaces after AGV implantation. It has been shown that IVB pre-treatment can effectively increase the complete success rate in NVG patients undergoing AGV implantation surgery.[14] Although the beneficial effect of Bevacizumab has been shown in neovascular glaucoma the exact role of this agent in other types of glaucoma has not been clarified.[14]

The aim of the current study was to compare the outcome of AGV implantation with and without adjunctive Bevacizumab injection.

METHODS

This prospective, randomized clinical trial was performed from April 2014 to July 2015 at Rassoul Akram Hospital, Iran University of Medical sciences, Tehran, Iran. Signed informed consent was obtained from all patients, and the ethical committee of the Iran University of Medical Sciences approved the study. Patients with intraocular pressure (IOP) more than 18 with full maximum tolerable medical treatment were considered as uncontrolled. Also patients with poor compliance to take medications were included.

Only patients with at least 6 months of follow-up were included in the statistical analysis.

The exclusion criteria were age younger than 18 years, those with active signs of ocular inflammation or NVG or being pregnant or currently breastfeeding. The eyes were randomly chosen by computer generated block randomization with block size of 4 and assigned to two groups: conventional AGV surgery (group 1) and surgery with sub conjunctival Bevacizumab injection (group 2). Before surgery, all participants underwent a comprehensive ophthalmic examination, including slit-lamp biomicroscopy, best-corrected visual acuity (BCVA), IOP measurement by a calibrated Goldmann applanation tonometer, dilated funduscopy with a 90-diopter lens and gonioscopy.

All surgeries were performed under either general or retrobulbar anaesthesia by discretion of two experienced surgeon (A.M. and N.N.).

We first performed fornix-based peritomy in the superotemporal quadrant. After priming of shunt (AGV FP7, New World Medical) with balanced salt solution, it was secured to the sclera with 8–0 nylon sutures 9–10 mm behind the limbus. The tube was trimmed bevelled up and then inserted into the anterior chamber. The tube was then secured to sclera with a 10–0 nylon suture and covered with a donor sclera. Conjunctiva was closed with 10/0 vicryl in a running fashion at the end.

In the same fashion, AGV implantation was performed in the Bevacizumab group, but after suturing the conjunctiva, 2.5 mg (0.1 mL) Bevacizumab was injected subconjunctivally at the site of the shunt plate using 30-gauge needle. All patients were treated with ciprofloxacin eye drops four times a day for 10 days and betamethasone eye drops every 2–4 h for 2 weeks that were tapered off slowly over 8–12 weeks. After surgery, antiglaucoma medications were added if necessary based on the targeted IOP. Patients were scheduled for seven postoperative follow-up visits within 6 months by assigned resident who was blind to the treatment group: days 1, 7, 14, 30, 60, 90 and 180. After month 6, follow-up visits were continued every 2 to 3 months. Success was defined in terms of IOP control based on two criterion: Criterion A: $5 \leq IOP < 22$ mmHg with at least ≥20% reduction in IOP without glaucoma medication (complete success), or with no more than two medications (qualified success). Cumulative success was defined as sum of complete and qualified success. Criterion B was defined according to criterion A but with IOP between 5 and 18 mmHg. Failure was defined when neither of the criteria was achieved, visual acuity became no light perception or reoperation was required.

Hypertensive phase was defined as IOP > 21 mmHg during the first 3 months after surgery after

reduction of IOP to less than 21 mmHg during the first postoperative weeks.

Statistical analysis

Normal distribution of data was assessed by Kolmogrov–Smirnov test and Q–Q plot.

Variables for statistical analysis included age, best corrected visual acuity, success rates, IOP and number of medications. Group sample sizes of 25 and 25 achieve 81% power to detect a IOP difference of 2.0 mmHg between the mean post of IOP with estimated group standard deviations of 2.5 and 2.4 and with a significance level (alpha) of 0.050 using a two-sided two-sample *t*-test. Statistical analysis was performed using SPSS software version 20. For the purposes of analysis, data were reported as mean (± SD) and frequency. The Student *t*-test or Mann–Whitney test was used to compare quantitative variables between two groups and Chi square test for categorical variables to determine statistical significance. Statistical significance was determined using a, two-tailed test *P* value ≤ 0.05.

RESULTS

A total of 50 patients were enrolled in this study. Ahmed valve was implanted in 25 eyes without Bevacizumab (Group 1) and in 25 eyes with subconjunctival Bevacizumab injection (group 2). All of the patients completed at least 6 months of follow-up period. Demographic characteristics of patients are summarized in Table 1. Both groups were comparable in terms of age, sex, initial BCVA, and number of glaucoma medications. Mean IOP at baseline was 24.88 ± 7.62 mmHg in group 1 and 27.52 ± 8.57 mmHg in group 2. ($P = 0.25$).

Mean follow-up was 10.32 ± 2.74 months in AVG alone and 10.56 ± 2.61 in Bevacizumab group ($P = 0.25$).

All patients had at least 6 months of follow-up and 18 patients in group 1 and 19 patients in group 2 had completed 1 year of follow-up. In all post-OP visits both groups showed statistically significant reductions in the mean IOP and in the mean number of glaucoma medications ($P < 0.05$)(Table 2) However, there was no significant difference between the two groups at each time point except at month 6 ($P = 0.04$). (Table 2) Number of medications decreased significantly from 3.32 ± 0.8 to 1.8 ± 0.9 in 1 year in group 1 and from 3.48 ± 0.65 to1.4 ± 0.9 in group 2 ($P < 0.00$) but there was no difference between the mean number of medications at each time points between two groups. (Table 3).

Mean IOP was lower in Bevacizumab group during 1 year of follow-up but the difference was significant only at month 6 ($P < 0.04$). (Table 3).

Tables 4 and 5 show the success rates based on criterion A and B in both group at 6th and 12th month visits.

The overall complication rate in both groups was 36%; the most common complication was shallow AC, which observed in six cases. Table 6 displays complication rates in each study group.

We had one case with choroidal detachment in each group and one case with wound dehiscence in group 2 which needed resuturing. No case of endophthalmitis, suprachorodal haemorrhage or plate exposure was observed. Hyphema was the most common complication in group 2 (four cases)

Table 1. Patient demographics

Characteristics		Group 1 (conventional AGV insertion)	Group 2 (AGV insertion with Bevacizumab)	*P* value
Age (mean ± SD) (years)		58.76 ± 12.11 Range: (25–73)	51.36 ± 15.44 Range: (21–81)	0.06
Sex (male %)		9/16	13/12	0.25
Laterality (right %)		52%	48%	0.77
Preoperative intraocular pressure (mmHg)		24.88 ± 7.62 Range: (13–40)	27.52 ± 8.57 Range: (16–46)	0.25
Preoperative number of medications		3.32 ± 0.80 Range: (2–4)	3.48 ± 0 0.65 Range (2–4)	0.545
Follow-up period (mean ± SD) (months)		10.32 ± 2.74 Range: (6–12)	10.56 ± 2.61 Range: (6–12)	0.25
Kind of glaucoma	Primary open angle glaucoma	52%	40%	0.42
	Chronic angle closure glaucoma	32%	28%	
	Pseudoexfoliation glaucoma	0%	12%	
	Congenital glaucoma	4%	0%	
	Post PK	0%	4%	
	Post vitrectomy	12%	16%	

PK, penetrating keratoplasty.

Table 2. Comparison of pre and postoperative characteristics in each group

Group	Characteristics	Preoperative	Postoperative (1 year)	*P* value
Group 1 (conventional AGV insertion)	Intraocular pressure (mmHg)	24.88 ± 7.62	15.72 ± 4.86	0.002
	Range (mmHg)	(13–40)	(10–27)	
	Antiglaucoma medications number	3.32 ± 0.80	1.82 ± 0.92	0.001
	Range	(2–4)	(0–2)	
	Best corrected visual acuity (logMAR)	0.94 ± 0.79	0.87 ± 0.83	0.67
Group 2 (AGV insertion with Bevacizumab)	Intraocular pressure (mmHg)	27.52 ± 8.57	14.00 ± 3.52	<0.001
	Range (mmHg)	(16–46)	(8–20)	
	Antiglaucoma medications number	3.48 ± 0.65	1.42 ± 0.90	<0.001
	Range	(2–4)	(0–3)	
	Best corrected visual acuity (logMAR)	0.85 ± 0.85	1.00 ± 1.11	0.31

Table 3. Comparison between two groups in terms of intraocular pressure and medications at each time points of follow-up[†]

Pre-OP medication	AGV (*n* = 25)	3.32 ± 0 0.80	0.545
	AGV + Bevacizumab (*n* = 25)	3.48 ± 0.65	
Post-OP 1 month medication	AGV (*n* = 25)	1.20 ± 1.04	0.32
	AGV + Bevacizumab (*n* = 25)	0.92 ± 0.95	
Post-OP 3 months medication	AGV (*n* = 25)	1.45 ± 0.85	0.812
	AGV + Bevacizumab (*n* = 25)	1.40 ± 0.85	
Post-OP 6 months medication	AGV (*n* = 25)	1.68 ± 0.80	0.310
	AGV + Bevacizumab (*n* = 25)	1.44 ± 0.86	
Post-OP 12 months medication	AGV (*n* = 18)	1.83 ± 0.92	0.167
	AGV + Bevacizumab (*n* = 19)	1.42 ± 0.90	
Pre-OP IOP	AGV (*n* = 25)	24.88 ± 7.62	0.25
	AGV + Bevacizumab (*n* = 25)	27.52 ± 8.57	
Post-OP 1 month IOP	AGV (*n* = 25)	18.20 ± 7.26	0.83
	AGV + Bevacizumab (*n* = 25)	17.80 ± 6.48	
Post-OP 3 months IOP	AGV (*n* = 25)	16.86 ± 3.68	0.07
	AGV + Bevacizumab (*n* = 25)	14.40 ± 5.09	
Post-OP 6 months IOP	AGV (*n* = 25)	16.52 ± 4.29	0.04+
	AGV + Bevacizumab (*n* = 25)	14.12 ± 4.0137	
Post-OP 12 months IOP	AGV (*n* = 18)	15.72 ± 4.86	0.22
	AGV + Bevacizumab (*n* = 19)	14.00 ± 3.52	

[†]*t*-test + significant.

Table 4. Success rates based on criterion a and B in Ahmed glaucoma valve implantation with or without subconjunctival Bevacizumab injection at 6 months

Criterion	Group	Complete success	Qualified success	Cumulative success	Failure
A	Group 1 (conventional AGV insertion)	12%	56%	68%	32%
	Group 2 (AGV insertion with Bevacizumab)	20%	72%	92%	8%
	P value (Chi square)	0.034			
B	Group 1 (conventional AGV insertion)	12%	40%	52%	48%
	Group 2 (AGV insertion with Bevacizumab)	20%	68%	88%	12%
	P value (Chi square)	0.005			

which resolved spontaneously in all cases. Hypertensive phase defined as IOP more than 21 mmHg when initial IOP after surgery has reached to less than 21 mmHg was observed in 60% in group 1 and 48% in group 2 during month 1 ($P = 0.39$) and in 24% and 12% in group 1 and 2 during month 1–3, respectively ($P = 0.39$). There was no significant association between IOP before surgery and the occurrence of hypertensive phase neither in first month nor in 3 months in both groups. ($P = 0.67$ and $P = 0.77$, respectively).

In multivariate regression analysis the only factor which has associated with failure was age in group 1 ($P < 0.03$). The main reasons for failure in group 1 with criterion A were: not reaching to less than 20% decrease in IOP (three cases), IOP more than 21 mmHg (two cases) and needing more than two antiglaucoma medications (two cases). In group two we had only one case of failure due to less than 20% decrease of IOP. We had no failure due to hypotony or secondary intervention.

Table 5. Success rate based on criterion A and B in Ahmed glaucoma valve implantation with or without subconjunctival Bevacizumab injection at 12 months

	Group	Complete success	Qualified success	Cumulative success	Failure
A	Group 1 (conventional AGV insertion)	11%	66.8%	77.8%	22.2%
	Group 2 (AGV insertion with Bevacizumab)	15.7%	73.8%	89.5%	10.5%
	P value (Chi square)	0.405			
B	Group 1 (conventional AGV insertion)	11%	60.2%	72.2%	27.8%
	Group 2 (AGV insertion with Bevacizumab)	15.7%	73.8%	89.5%	10.5%
	P value (Chi square)	0.232			

Table 6. Type of complications after Ahmed glaucoma valve implantation with or without subconjunctival Bevacizumab injection

Complications	AGV	AGV + Bevacizumab	Total
Shallow AC	3	3	6
Choroidal detachment	2	0	2
Hypotonia	2	2	4
Hyphema	0	4	4
Leakage	0	1	1
Malignant glaucoma	0	1	1
Implant extrusion	0	0	0
Endophthalmitis	0	0	0
Total	7 (28%)	11 (44%)	18 (36%)

DISCUSSION

Glaucoma is the second cause of blindness in the world[15] and trabeculectomy and tube shunt surgery are the most common surgical procedure for management of glaucoma. The efficacy of a shunt surgery and the level of IOP depends largely on the consistency and permeability of capsule around the plate of drainage device.[16] The formation of thick vascularized fibrous capsule around the shunt decreases the filtration which may lead to surgical failure.[7] The rate of success after AGV implantation is different and depends on the study design and type of glaucoma. But on average approximately 50% of single-plate AGV implantations in refractory glaucoma were considered successful after 5 years of follow-up.[1,17]

Antimetabolite application can significantly inhibit fibrosis, and is widely used in drainage and fistulizing glaucoma surgeries.[3,4] However, several studies in the short and medium term follow-up have showed ineffectiveness of mitomycin C in improving success rate of shunt implantation both in the short and in medium term follow-up.[5,6]

The purpose of this study was to evaluate the effect of Bevacizumab as an antiangiogenic factor adjunctive to AGV in controlling IOP in a randomized clinical trial. VEGF stimulates multiple components of the wound-healing cascade, such as angiogenesis, collagen deposition and epithelization,[8–11] all of them are important risk factor for failure of glaucoma filtration surgeries.

Bevacizumab was approved by the US Food and Drug Administration (FDA) in 2005 for the treatment of colorectal and breast cancers, but it has also been used as an off label treatment in several ocular conditions such as neovascular glaucoma, age-related macular degeneration and diabetic retinopathy.[18]

Different routes of administration have been proposed for application of anti-VEGF agents. Subconjunctival administrations offer direct modulation of the conjunctival wound-healing process. In general, after subconjunctival injection some of the Bevacizumab may penetrate to intraocular tissues including the retina/choroid, iris/ciliary body, and vitreous via the sclera and also some is cleared via conjunctival blood and lymphatic flow.[19]

After subconjunctival injection of Bevacizumab, the scleral matrix may work as a depot and causes longer duration of exposure of iris, ciliary body to this substance.[19]

Although optimal dose of subconjunctival Bevacizumab is not clear, injections containing 1.25 or 2.5 mg of drug were most commonly employed.[17,18,20]

We used subconjunctival Bevacizumab after finishing shunt surgery and evaluated the effect of this antiangiogenic agent on survival of shunt surgery during the first year of follow-up.

We observed lower IOP in Bevacizumab injection group during follow up but the difference between mean IOP was not significant except at month 6 in favour of Bevacizumab group. We observed 68% cumulative success with Criteria A (IOP <21 mmHg) on cases without injection and 96% in injection group ($P < 0.04$). This demonstrates that Bevacizumab enhances the survival in Ahmed valve in our series in 1 year of follow-up. We considered criteria B as IOP < 18 mmHg and found 60% cumulative success in group 1 and 96% in group 2, respectively ($P < 0.01$). Our success rate in Bevacizumab group was a bit higher than previous published studies.[21–23]

We found better success rate and lower number of antiglaucoma medications in group 2 with Bevacizumab injection. We observed hypertensive phase in 68% patients without injection of Bevacizumab and 52% of patients with injection ($P > 0.05$%), which is not different from to previous published studies.[17,24,25]

Meanwhile we found no correlation between hypertensive phase and age, sex and type of glaucoma.

There were a few studies, which evaluated the role of subconjunctival Bevacizumab in shunt surgery. To the best of our knowledge it is the first randomized clinical trial comparing AGV implantation with Bevacizumab with standard implantation surgery in adult patients.

We found only one study which compared role of subconjunctival Bevacizumab in shunt surgery in paediatric glaucoma. In this study Mahdy used 1.25 mg of Bevacizumab subconjunctivally around the valve body of Ahmed valve after finishing surgery and compared it with mitomycin and placebo. He observed 80% total success in first group in which Bevacizumab augmented Ahmed valve was carried out in comparison with 90% total success in group II in which MMC was used during Ahmed valve implantation and this difference was not significant ($P > 0.05$), but in group III in which Ahmed valve implanted without adjuvant, total success was 60% with no cases of qualified success, and this difference was statistically significant to both groups I and II ($P < 0.05$).

They concluded that both mitomycin and Bevacizumab could increase valve survival but Bevacizumab was safer compared to mitomycin.[26]. Rojo-Arnao in a pilot study used subconjunctival Bevacizumab in seven patients with Ahmed valve and compared it with six case of shunt insertion without injection. They showed that use of Bevacizumab after 3 months can cause less hypertensive phase after Ahmed valve surgery.[27] In our study the rate of hypertensive phase was not significantly different between two groups.

We observed only one case of leakage in Bevacizumab group, which needed resuturing. We used only non-absorbable sutures and no case of exposure or protrusion occurred in 1 year.

In spite of increase in number of tube surgery there is no absolute way for prevention of excessive fibrosis around the device yet. Although use of antifibrotic agents decrease scar formation after trabeculectomy the same effect has not been documented after shunt surgery. Costa *et al.*, in study on 60 patients with refractory glaucoma used intraoperative MMC (0.5 mg/mL for 5 min) ($n = 34$) or balanced salt solution ($n = 26$) during Ahmed glaucoma valve implantation. After mean follow-up of 12.3 months Mitomycin C did not increase the short- or intermediate-term success rates of Ahmed glaucoma valve implantation.[6] Yazdani *et al.*, in a randomized clinical trial compared Ahmed valve implantation alone with Mitomycin and wrapping of Ahmed valve in a amniotic membrane as an anti-inflammatory agent and found no influence from MMC and amniotic membrane on success rate and hypertensive phase.[28] Since fibroblast function and growth of new vessels is a component of healing of the bleb, there have been attempts to retard this healing by the use of Bevacizumab.

Li *et al.* reported that administration of Bevacizumab significantly inhibited VEGF-induced Tenon fibroblast proliferation in human ($P = 0.04$).[7] Memarzadeh *et al.* showed that in the rabbit subconjunctival injection of Bevacizumab could increase bleb survival.[29] If we accept that VEGF directly stimulates both vascular endothelial cells and fibroblasts and may be the link between angiogenesis and scar formation, inhibition of VGEF can reduce fibrosis and may improve the success rates of the shunt surgery in future.

As it mentioned previously our trial showed better results with Bevacizumab than classic surgery. The strength of the present analysis is that it is a randomized clinical trial with direct comparison of study groups.

We had limitations in our study. Small number and heterogeneous type of study subjects and short term of follow-up were among the main limitations of this study. So more clinical trials with long term follow-ups are necessary to show exact role of Bevacizumab in shunt surgery. In conclusion it seems that Bevacizumab may lead to lower IOP and better success during the first year of surgery.

References

1. Gedde SJ, Singh K, Schiffman JC, Feuer WJ, Tube Versus Trabeculectomy Study Group. The tube versus trabeculectomy study: interpretation of results and application to clinical practice. *Curr Opin Ophthalmol* 2012; **23**: 118–26.
2. WuDunn D, Cantor LB, Palanca-Capistrano AM *et al.* A prospective randomized trial comparing intraoperative 5-fluorouracil vs. mitomycin C in primary trabeculectomy. *Am J Ophthalmol* 2002; **134**: 521–8.
3. Beckers HJ, Kinders KC, Webers CA. Five-year results of trabeculectomy with mitomycin C. *Graefes Arch Clin Exp Ophthalmol* 2003; **241**: 106–10.
4. Casson R, Rahman R, Salmon JF. Long term results and complications of trabeculectomy augmented with low dose mitomycin C in patients at risk for filtration failure. *Br J Ophthalmol* 2001; **85**: 686–8.
5. Cantor L, Burgoyne J, Sanders S, Bhavnani V, Hoop J, Brizendine E. The effect of mitomycin C on Molteno implant surgery: a 1-year randomized, masked, prospective study. *J Glaucoma* 1998; **7**: 240–6.
6. Costa VP, Azuara-Blanco A, Netland PA, Lesk MR, Arcieri ES. Efficacy and safety of adjunctive mitomycin C during Ahmed glaucoma valve implantation: a prospective randomized clinical trial. *Ophthalmology* 2004; **111**: 1071–6.

7. Li Z, Van Bergen T, Van de Veire S *et al*. Inhibition of vascular endothelial growth factor reduces scar formation after glaucoma filtration surgery. *Invest Ophthalmol Vis Sci* 2009; **50**: 5217–25.
8. Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. *Surv Ophthalmol* 2003; **48**: 314–46.
9. Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. *Microsc Res Tech* 2003; **60**: 107–14.
10. Asahara T, Bauters C, Zheng LP *et al*. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. *Circulation* 1995; **92**: II365–71.
11. Sun XT, Ding YT, Yan XG *et al*. Angiogenic synergistic effect of basic fibroblast growth factor and vascular endothelial growth factor in an in vitro quantitative microcarrierbased three dimensional fibrin angiogenesis system. *World J Gastroenterol* 2004; **10**: 2524–8.
12. Zhou M, Chen S, Wang W *et al*. Levels of erythropoietin and vascular endothelial growth factor in surgery-required advanced neovascular glaucoma eyes before and after intravitreal injection of bevacizumab. *Invest Ophthalmol Vis Sci* 2013; **54**: 3874–9.
13. Nissen NN, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro L. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol* 1998; **152**: 1445–52.
14. Zhou M, Xu X, Zhang X. Clinical outcomes of Ahmed glaucoma valve implantation with or without intravitreal bevacizumab pretreatment for Neovascular glaucoma: a systematic review and meta-analysis. *J Glaucoma* 2016; **25**: 551–7.
15. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006; **90**: 262–7.
16. Minckler DS, Shammas A, Wilcox M, Ogden TE. Experimental studies of aqueous filtration using the Molteno implant. *Trans Am Ophthalmol Soc* 1987; **85**: 368–92.
17. Souza C, Tran DH, Loman J, Law SK, Coleman AL, Caprioli J. Long-term outcomes of Ahmed glaucoma valve implantation in refractory glaucomas. *Am J Ophthalmol* 2007; **144**: 893–900.
18. Grisanti S, Ziemssen F. Bevacizumab: off-label use in ophthalmology. *Indian J Ophthalmol* 2007; **55**: 4.
19. Nomoto H, Shiraga F, Kuno N *et al*. Pharmacokinetics of bevacizumab after topical, subconjunctival, and intravitreal administration in rabbits. *Invest Ophthalmol Vis Sci* 2009; **50**: 4807–13.
20. Nilforushan N, Yadgari M, Kish SK, Nassiri N. Subconjunctival bevacizumab versus mitomycin C adjunctive to trabeculectomy. *Am J Ophthalmol* 2012; **153**: 352–7.
21. Gil-Carrasco F, Salinas-VanOrman E, Recillas-Gispert C, Paczka JA, Gilbert M, Arellanes-García L. Ahmed valve implant for uncontrolled uveitic glaucoma. *Ocular Immunol Inflam* 1998; **6**: 27–37.
22. Kook M, Yoon J, Kim J, Lee MS. Clinical results of Ahmed glaucoma valve implantation in refractory glaucoma with adjunctive mitomycin C. *Ophthalmic Surg Lasers* 2000; **31**: 100–10.
23. Ayyala RS, Zurakowski D, Smith JA *et al*. A clinical study of the Ahmed glaucoma valve implant in advanced glaucoma. 1998; **105**: 1968–Ophthalmology, 76.
24. Pakravan M, Esfandiari H, Yazdani S *et al*. Mitomycin C-augmented trabeculectomy: subtenon injection versus soaked sponges: a randomised clinical trial. *Br J Ophthalmol* 2017; **0**: 1–6.
25. Nouri-Mahdavi K, Caprioli J. Evaluation of the hypertensive phase after insertion of the Ahmed glaucoma valve. *Am J Ophthalmol* 2003; **136**: 1001–8.
26. Mahdy RA. Adjunctive use of bevacizumab versus mitomycin C with Ahmed valve implantation in treatment of pediatric glaucoma. *Glaucoma* 2011; **20**: 458–63.
27. Rojo-Arnao M, Albis-Donado OD, Lliteras-Cardin M, Kahook MY, Gil-Carrasco F. Adjunctive bevacizumab in patients undergoing Ahmed valve implantation: a pilot study. *Ophthalmic Surg Lasers Imaging* 2011; **42**: 132–7.
28. Yazdani S, Mahboobipour H, Pakravan M, Doozandeh A, Ghahari E. Adjunctive mitomycin C or amniotic membrane transplantation for Ahmed glaucoma valve implantation: a randomized clinical trial. *J Glaucoma* 2016; **25**: 415–21.
29. Memarzadeh F, Varma R, Lin LT *et al*. Postoperative use of bevacizumab as an antifibrotic agent in glaucoma filtration surgery in the rabbit. *Invest Ophthalmol Vis Sci* 2009; **50**: 3233–7.